# *Vibrio* in Stinging Seaweed: Potential Infection

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*Toxic strains of the finely filamentous, velvety, dark-olive green to black algal organism,* Microcolus Lyngbyaceus, *(formerly* Lyngbya majuscula *Gomont, or "lyngbya") have been recognized as etiologic agent of "stinging seaweed" dermatitis (one of several forms of "swimmer's itch") in Hawaii since the late 1950s as reviewed[1]. Lymphadenopathy, pustular folliculitus, and local infections have been reported in some persons[1,2].*

## Introduction

In 1959 it was reported that a gram-negative pigment-producing bacillus was the predominant bacterium isolated from marine *Lyngbya majuscula*[1,2]. In 1961 it was reported that a nonhemolytic streptococcus was cultured from the aspirated contents of a scrotal vesicle in a stinging seaweed dermatitis patient[2]. That same report mentioned that neither *Candida albicans* nor dermatophytes were identified. We describe the results of culture and sensitivity determinations for specimens of *Microcoleus lyngbyaceus* algae collect ed during a toxic algal outbreak.

## Materials and Methods

Specimens of organisms tentatively thought to be *Microcoleus lyngbyaceus*[4] tentatively were obtained during an outbreak of "stinging seaweed" dermatitis at Kailua Beach, Oahu, Hawaii, on September 8, 1979 and were examined by light microscopy as fresh- and formalin-fixed material.

A representative of the Hawaii Department of Health Pollution Investigation and Enforcement Branch obtained surface seawater specimens in sterile containers approximately 50 yards off-shore on February 15, 1984, from areas on Maui labeled the Lahainaluna area and the Lahaina Broiler area. Undiluted (0.1 ml) and diluted (1:100) samples of seawater were plated directly onto marine agar. A specimen of *Microcoleus lyngbyaceus* obtained on February 4, 1984 from the south side of Lahaina near the Puamana subdivision was streaked directly onto marine agar.

The marine agar plates (kept at 26° to 27°c) were examined at 24- hours, 48-hours, and 1-week of incubation for signs of growth. Representative bacterial colonies on the marine agar plates were sampled and gram-stained. Bacterial colonies of gram-negative rods were then inoculated onto triple sugar-iron agar with 50% seawater while colonies comprised of gram-positive rods were inoculated onto tryptose broth made with 50% seawater. Seawater (0.01 ml) and seaweed specimens also were cultured on Sabouraud dextrose agar and incubated at 25° to 27°c for 2 weeks in order to isolate streptomyces. Culture isolates then were characterized using physical/biochemical identification tests and antibiotic sensitivity tests.

Since initial screening of seawater and *Microcoleus lyngbyaceus* algal specimens revealed indole-positive vibrios and *Vibrio alginolyticus*, more samples from areas endemic for stinging seaweed dermatitis and/or stinging seaweed were obtained. *M. lyngbyaceus* algal specimens and seawater specimens were obtained from the same area of south Lahaina, Maui (ie, south of Lahaina Shores near the Puamana subdivison) on September 1,1984, and from the Anini Beach area near Hanalei Bay on the island of Kauai on September 3,1984. The Lahaina shorebreak seawater salinity was 14 parts per thousand (ppt) and the Kauai shorebreak seawater specimen salinity was 13 ppt; whereas Hana, Maui, freshwater stream control sample measured 0.2 ppt, as measured by conductivity methods.

The specimens for culture were serially diluted with phosphate-buffered saline and plated onto marine agar and TCBS (thiosulfate-citrate-bile salts-sucrose), a medium selective for vibrio bacteria. All cultures were incubated at 37°C. The plates were examined for bacterial growth at 24 and 48 hours. Representative bacterial colonies on the plates were sampled, gram-stained, and characterized using the API 20E identification system. Indole-positive vibrios were selected and further characterized as isolates of indole-positive (IND+) and tryptophan-deaminase negative (TDA negative) suggesting the presence of indoles and not free tryptophans (eg, *lyngbya*-toxin-A and/or lyngbyatoxin-A precursors).

On September 7, 1985, a specimen of seawater and algae consistent with *M. lyngbyaceus* was collected from surface water at the Kahala Beach ocean shoreline on Oahu from which *Vibrio alginolyticus* was recovered.

## Results

The baterium *Vibrio alginolyticus* was cultured from marine specimens of the blue-green algal organism *Microcoleus Lyngbyaceus* obtained from 3 endemic areas of toxic stinging seaweed, namely Anini Beach on Kauai, Lahaina, Maui and Kahala Beach, Oahu. *Vibrio alginolyticus* was cultured from *M. lyngbyaceus* in seawater as well as from moist *M. lyngbyaceus* recovered from the beach (ie, as beach wash) at Lahaina, Maui. The *Vibrio alginolyticus* recovered was a gram-negative rod that formed yellow mucoid colonies on TCBS agar. The *V. alginolyticus* was sensitive to conservative antibiotic activity of

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chloramphenicol, gentamicin, tetracycline, sulfadiazine, and trimethoprim/sulfamethxazole.

## Discussion

In 1959 it was reported that a "gram-negative pigment producing bacillus" was isolated from *Lyngbya majuscula* Gomont[3]. Herein is described the identification of *Vibrio alginolyticus*, a gram-negative rod, from specimens of toxic *Microcoleus lyngbyaceus*. It also has been shown that brackish water can have *Vibrio parahemolyticus* on and/or in the *M. lyngbyaceus* algae. Both *V. alginolyticus* and *V. parahemolyticus* histochemically and histologically stain positive for indoles-gram-positive, gram-negative cocci and rods being seen on the algae using Brown and Brenn stains on formalin-fixed paraffin block *M. lyngbyaceus* algae. It is noteworthy that the February 15, 1984 Lahainaluna seawater grew *Moraxella sp*, a group c M-6 Moraxella-like bacterium, and *Pseudomonas diminuta*; February 15, 1984 Lahaina Broiler area seawater grew gr. M-6 Moraxella-like bacteria, *Providencia sp*, *Vibrio sp*, and *Pseudomonas diminuta*; February 4,1984 Lahaina Puamana *M. lyngbyaceus* grew *Vibrio alginolyticus*; whereas September 1, 1984 Lahaina, Maui Puamana seawater and *M. lyngbyaceus* grew *Vibrio alinolyticus* and *Vibrio sp.*

Although in one report[2] it was mentioned that neither *Candida albicans* nor dermatophytes were encountered in a stinging seaweed patient, one species of fungus was cultured from the Kahaluu edge of Kaneohe Bay, Oahu; it came from algal *M. lyngbyaceus*, possibly nontoxic and/or slightly toxic. The fungus was seen as yeast forms within an algal filament, and on culture was black with yeast forms and septated, jointed hyphae. The fungal hyphae were heavily indole-positive to indole histochemistry and indole histological straining. The tips of the Kaneohe Bay *M. lyngbyaceus* algae were faintly indole-positive, suggesting the possible presence of lyngbyatoxin A, aplysiatoxin and debromoaplysiotoxin are 3 tumor-producing substances described in toxin of *Microcoleus lyngbyaceus*—co-carcinogens, or phase II tumor/cancer promoters.

*Vibrio parahemolyticus*, and more so *Vibrio vulnificus* can produce blisters, which occurred in a number of patients with *lynbya* stinging seaweed dermatitis. Arsenic occurs in *lyngbya* also.

Testing for antimicrobial/antibiotic sensitivity indicated that *Vibrio alginolyticus* is not uniformly sensitive to all antibiotics; however, there appeared to be conservative sensitivity of *V. alginolyticus* to antibiotics listed under results.

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